THE EXTENDED ELUTION BY CHARACTERISTIC POINT METHOD TO DETERMINE ADSORPTION ISOTHERMS OF LABYRINTHOPEPTIN FOR PURIFICATION VIA ION-EXCHANGE CHROMATOGRAPHY

Jonas Lohr[1*], Dave Hartig[2*], Stephan Scholl[2], Antje Spieß[1]

[1] Technische Universität Braunschweig, Institute of Biochemical Engineering, Rebenring 56, 38106 Braunschweig

[2] Technische Universität Braunschweig, Institute for Chemical and Thermal Process Engineering, Langer Kamp 7, 38106 Braunschweig

[*]both authors contributed equally

ABSTRACT

Ion-exchange chromatography (IEC) is the most frequently used technique to purify proteins and therefore, plays an important role in process development for therapeutical proteins. To improve the purification using ion-exchange chromatography, adequate characterization of adsorption isotherms is obligatory. The Elution by Characteristic Point method (ECP) can be used to determine adsorption isotherms applying only minor amounts of sample material to the chromatography column. Here, the applicability of the extended ECP method to determine adsorption isotherms of the model protein bovine serum albumin (BSA) using bovine hemoglobin (bHb) as tracer substance to quantify all non-idealities of the system is shown. The resulting isotherm was validated using the static batch approach. In the next step, the gained knowledge is used to measure isotherms of Labyrinthopeptins A1 and A2, which show promising activity against retroviruses like *herpes simplex virus* or *human immunodeficiency virus*.

Keywords: Adsorption isotherm, Elution by Characteristic Point, Ion-Exchange Chromatography, Labyrinthopeptin

INTRODUCTION

The market for biopharmaceuticals is still growing, reaching the highest number of new approvals since 1996 in 2017 [Morrison, 2018]. In the same year sales of biopharmaceuticals reached 188 billion US$ with monoclonal antibodies alone combining for 65.6% of total sales [Morrison, 2018; Walsh, 2018].

General production schemes for biopharmaceuticals consist of the production of the target product using mammalian cells or microorganisms and subsequent downstream processing. Chromatography is the most frequently used technique for the purification of biopharmaceuticals. For industrial applications, 45% of all chromatographic purification steps are ion-exchange chromatography steps [Karlsson, 2011]. This implies the important role IEC plays in the production of biopharmaceuticals. However, chromatographic purification processes are still expensive making downstream processing with up to 80% of the overall process costs the most cost-intensive part in the production of biopharmaceuticals [Roque, 2004]. Hence, one should focus on finding efficient and therefore economic operation points for a specific chromatographic separation task.

Lanthipeptides, post-translationally modified peptides with a characteristic polycyclic structure, are such a promising group of biopharmaceuticals showing high potential against retroviruses *in vitro* [Meidl, 2010], but with large differences in their potential [Férir, 2013]. For example, Labyrinthopeptin A1 is 10-fold more potent against *herpes simplex virus* and *human immunodeficiency virus* than Labyrinthopeptin A2 making a separation of these peptides desireable. IEC is a very promising separation technique as shown for nisin, another prominent lanthipeptide [Abts, 2010].

A deep understanding of the interaction between peptides and stationary phase under various process conditions is needed to account for variations in the fermentation broth fed into the downstream processing. Hence, the characterization of adsorption isotherms is

https://doi.org/10.24355/dbbs.084-202001221252-0

essential for process development. While a variety of methods has been described in literature to determine adsorption isotherms experimentally, only the dynamic so-called Elution by Characteristic Point (ECP) method offers the possibility to gain complete isotherm data with just one experiment leading to smallest consumption of substance [Seidel-Morgenstern, 2004]. This low demand is a crucial advantage as the targeted peptides A1 and A2 are not commercially available but have to be produced and purified specifically for adsorption experiments. One of the most limiting factors for ECP, especially in early stage development, is the need for a high number of theoretical stages to provide accurate isotherm data. This limitation was recently overcome by an Extended Elution by Characteristic Point (EECP) method, where non-idealities of the system, *e.g.,* convection, dispersion or low number of theoretical plates are quantified by using a specific marker substance [Hartig 2015].

In this work, EECP is transferred to the system of labyrinthopeptins and ion-exchange resins. To this end, the cheap and easy available proteins BSA and bHb are used as model substances to characterize the required process conditions to generate adsorption isotherms and to understand occurring problems and limitations. Then, the knowledge is used to measure adsorption isotherms of labyrinthopeptins on ion-exchange resins.

THEORY

This section gives a brief summary of the derivation and use of EECP [Hartig, 2015]. The standard equilibrium model of chromatography neglects dispersion and assumes instantaneous adsorption equilibrium between the bulk fluid phase and the particle at every axial position. While the equilibrium assumption can be fulfilled by using low flow rates and small particle diameters, neglecting the axial dispersion leads to the need of a high number of theoretical plates in classical ECP. In contrast, EECP method assumes that all non-idealities, *e.g.*, non-rectangular injection profile and axial dispersion, are linearly independent from the effects of adsorption and can be determined by a tracer injection. Hence, all non-ideal influences are lumped into a concentration-dependent system dead volume V_S that is subtracted from the retention volume of the adsorptive V_R. Then, the loading is calculated using this corrected retention volume ($V_R - V_S$):

$$q(c_i) = \frac{c_{i,max}}{m_{Ad}} \int_0^{c_i^\circ} \left(V_R(\tilde{c}_i^\circ) - V_S(\tilde{c}_i^\circ) \right) d(\tilde{c}_i^\circ) \qquad (1)$$

$$c_i^\circ = \frac{c_i}{c_{i,max}} \quad c_j^\circ = \frac{c_j}{c_{j,max}} \qquad (2)$$

It is worth stressing that c_i° and c_j° represent the normalized concentrations of the adsorbing and the tracer substance, respectively. Both concentration profiles are normalized for comparability.

RESULTS AND DISCUSSION

As discussed in the last section, the application of EECP requires the flow rate to be small enough to allow for an establishment of equilibrium between bulk liquid and stationary phase. Hence, Fig. 1 shows the influence of the superficial velocity on the determined Henry coefficient for BSA vs bHb defined by:

$$H = \frac{V_{BSA} - V_{bHb}}{m_{Ad}} \qquad (3)$$

The superficial velocity was corrected for a decreased available cross section due to partial blocking of the bed as determined by total porosity measurements using glucose as tracer (see [Hartig, 2017] for further details on porosity measurement). It becomes obvious that the superficial velocity should not exceed 10 mm/min to allow neglecting kinetic effects.

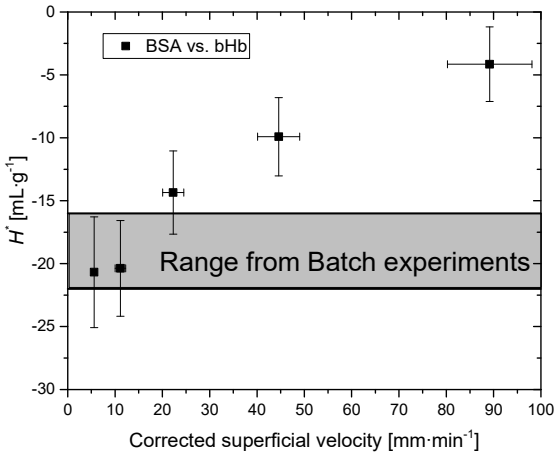

Figure 1: Henry coefficient between BSA and bHb on Q Sepharose FF at 25 °C in 20 mM sodium phosphate buffer (ph 7) determined by retention time of peak maximum at varying superficial velocity. Concentration of BSA and bHb was approximately 7 µM and 10 µM, respectively. Volumetric flow was varied between 25 µL/min and 400 µL/min. Data from [Hartig, 2017].

Besides the use of a feasible small flow rate, two additional factors had to be included to determine BSA isotherms. First, only 35% of the expected total porosity were measured leading to a correction of the mass of adsorbent to only 35% of the mass weighted into the column. Second, bHb showed a measureable

https://doi.org/10.24355/dbbs.084-202001221252-0

adsorption at the applied conditions. Hence, the slope of the bHb isotherm was determined in batch experiments (data not shown) and this slope was added to the BSA isotherm. With these corrections, the adsorption isotherms of BSA were determined. This is exemplary shown at 25 °C in Fig. 2.

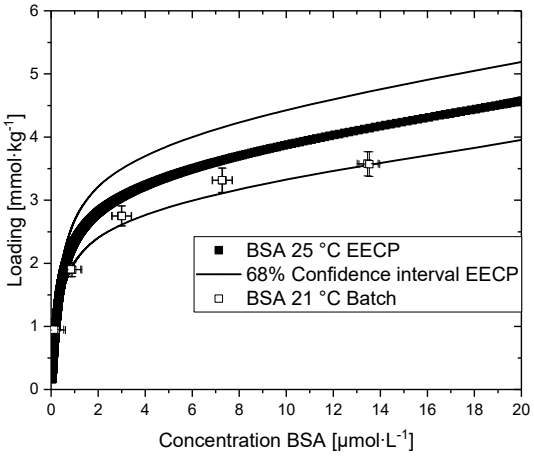

Figure 2: Adsorption isotherm of BSA at 25 °C in 20 mM sodium phosphate buffer (ph 7), bHb was used as tracer. Data from [Hartig, 2017].

A good agreement between the results of batch and EECP measurements can be seen. Size exclusion effects that could not be totally ruled out due to the different shape of BSA and bHb might cause the small deviation at higher concentrations. This stresses the importance to find feasible tracers for the particular adsorptive. With respect to used material, static adsorption experiments needed about 37 mg BSA whereas one EECP isotherm was measured with only 12 mg BSA. Although the calibration of the detector and the determination of a feasible flow rate lead to an additional consumption of about 25 mg BSA, recalibration is not necessary for the investigation of other conditions like different buffer concentrations or temperatures. Overall, EECP might reduce the material needed by nearly 70% for all further isotherms compared to batch.

The behavior of the small peptides Labyrinthopeptin A1 and A2, having molecular masses of 2.072 kDa and 1.922 kDa, respectively, might differ notably from the larger protein BSA (66.5 kDa) due to increased diffusion coefficient but also increased diffusion path inside the porous matrix. Hence, adjustment of the experimental conditions is mandatory to fulfill the equilibrium assumption before conducting EECP measurements. As indicated in Fig. 3, superficial velocities below 10 mm/min should be chosen for Labyrinthopeptin A1 to ensure establishment of equilibrium. It is worth noting that the similar value compared to BSA should rather be seen as coincidence since the impact of smaller molecule size on diffusion kinetic is complex. The retention coefficient can be calculated using the retention volume at the peak maximum:

$$k' = \frac{V_R - V_T}{V_T} \quad (4)$$

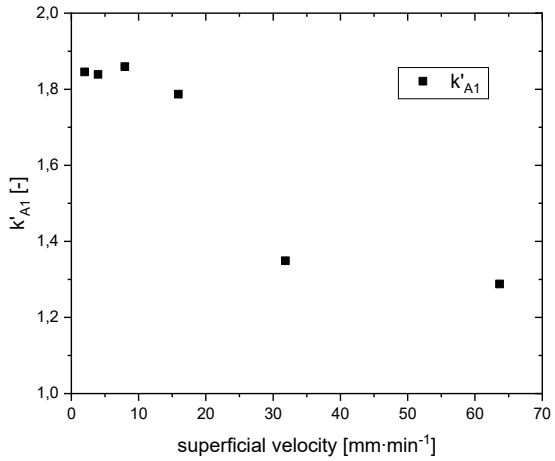

Figure 3: Retention coefficient of Labyrinthopeptin A1 on Q Sepharose FF at 25 °C in 20 mM Bis-Tris buffer (ph 7) with 200 mM sodium chloride at varying superficial velocities.

CONCLUSION AND OUTLOOK

The extended Elution by Characteristic Point method is a rapid approach to determine adsorption isotherms consuming only small amounts of sample material. This was successfully shown for model proteins BSA and bHb on Q Sepharose FF, where a good correlation to the well-established batch method can be seen.

Since the method has been established for the model proteins BSA and bHb and crucial process parameters for the determination of adsorption isotherms for labyrinthopeptins on Q Sepharose FF have been characterized, isotherm data for A1 and A2 will be generated in future experiments.

NOMENCLATURE

c	concentration, mg/L
k’	retention coefficient, 1
m	mass, mg

https://doi.org/10.24355/dbbs.084-202001221252-0

q	loading of adsorbent, mg/mg
V	volume, mL
BSA	bovine serum albumin
bHb	bovine hemoglobin
ECP	elution by characteristic point
IEC	ion-exchange chromatography

SUBSCRIPT

°	normalized
0	initial
A1	Labyrinthopeptin A1
Ad	adsorbent
i	solute
j	marker
max	maximum
R	retention
T	total

REFERENCES

[Abts, 2018] Abts, A., Mavaro, A., Stindt, J., Bakkes, P. J., Metzger, S., Driessen, A. J., Smith, S. H. M., & Schmitt, L. (2011). Easy and rapid purification of highly active nisin. *International Journal of Peptides,* 2011.

[Férir, 2013] Férir, G., Petrova, M. I., Andrei, G., Huskens, D., Hoorelbeke, B., Snoeck, R., Vanderleyden, J., Balzarini, J., Bartoschek, S., Brönstrup, M., Süssmuth, R. D., & Schols, D. (2013). The lantibiotic peptide labyrinthopeptin A1 demonstrates broad anti-HIV and anti-HSV activity with potential for microbicidal applications. *PloS One,* 8(5), e64010.

[Hartig 2015] Hartig, D., Waluga, T., & Scholl, S. (2015). Expanding the elution by characteristic point method to columns with a finite number of theoretical plates. *Journal of Chromatography A*, *1413*, 77-84.

[Hartig, 2017] Hartig, D., Waluga, T., Schmidt, C., Fieg, G., & Scholl, S. (2017). Extended elution by characteristic point method for characterization of protein ion-exchange adsorption. *Chemical Engineering & Technology*, *40*(10), 1940-1945.

[Karlsson, 2011] Karlsson, E., & Hirsh, I. (2011). Ion exchange chromatography. *Protein Purification: Principles, High Resolution Methods, and Applications (Vol. 151), John Wiley & Sons,* 93-134.

[Meindl, 2010] Meindl, K., Schmiederer, T., Schneider, K., Reicke, A., Butz, D., Keller, S. & Brönstrup, M. (2010). Labyrinthopeptins: a new class of carbacyclic lantibiotics. *Angewandte Chemie International Edition*, 49(6), 1151-1154.

[Morrison, 2018] Morrison, C. (2018). Fresh from the biotech pipeline--2017. *Nature Biotechnology, 36(2)*, 131-137.

[Roque, 2004] Roque, A. C. A., Lowe, C. R., & Taipa, M. Â. (2004). Antibodies and genetically engineered related molecules: production and purification. *Biotechnology Progress, 20(3),* 639-654.

[Seidel-Morgenstern, 2004] Seidel-Morgenstern, A. (2004). Experimental determination of single solute and competitive adsorption isotherms. *Journal of Chromatography A*, *1037*(1-2), 255-272.

[Walsh, 2018] Walsh, G. (2018). Biopharmaceutical benchmarks 2018. *Nature Biotechnology*, *36*, 1136-1145

https://doi.org/10.24355/dbbs.084-202001221252-0